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Orphan nuclear receptor COUP-TFII plays important roles in controlling diverse aspects of cell growth, development, differentiation and homeostasis. During the development, its expression is spatially and temporally restricted within mesenchymal cells of many organs (including developing prostate glands) that require interactions between the mesenchymal and epithelial compartments for proper development. The timing and expression pattern of COUP-TFII in prostate are closely correlated with the development and differentiation of the prostate glands, suggesting that COUP-TFII is an important modulator for mesenchymal-epithelial interactions required for the normal prostate development and tumorgenesis of prostate cancer. To study COUP-TFII's function during the development of prostate gland and the carcinogenesis of prostate cancer, we have successfully generated adenoviral vectors that effectively express sense and antisense COUP-TFII mRNA. More effort is being devoted to analyze the effect of COUP-TFII over- or under-expression on the ability of mesenchyme to induce epithelial differentiation, and the ability of tumorigenesis of prostate cancer cell lines. In addition, we established rUGM cell lines which over- and under-express COUP-TFII. The effect of changing the level of COUP-TFII expression in rUGM cells on the modulation of tumor growth induced by epithelial cells in asthymic nude mice will be studied.

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Introduction

Mesenchymal-epithelial interactions play pivotal roles in the regulation of growth and function of prostate epithelial cells. Prostate epithelial differentiation is dictated by its surrounding mesenchymal cells, which determine androgen induced growth responsiveness and the expression of specific secretory proteins in normal prostate gland. During neoplastic progression, organ specific mesenchymal cells have been shown to determine the rate of prostate tumor growth, differentiation and androgen responsiveness (1-4). These indicate the importance of the paracrine signal(s) produced by mesenchyme in determining the cell fate and differentiation of nearby prostate epithelial cells. Orphan nuclear receptor COUP-TFII, a transcription factor that belongs to the steroid/thyroid hormone receptor superfamily, plays important roles in controlling diverse aspects of cell growth, development, differentiation and homeostasis. During the development, its expression is spatially and temporally restricted within mesenchymal cells of many organs (including developing prostate glands) that require interactions between the mesenchymal and epithelial compartments for proper development. The timing and expression pattern of COUP-TFII in prostate are closely correlated with the development and differentiation of the prostate glands. This strongly suggests that COUP-TFII is an important modulator not only for mesenchymal-epithelial interactions, but also for normal prostate development as well as tumorgenesis of prostate cancer. To study COUP-TFII's function during the development of prostate gland and carcinogenesis of prostate cancer, we propose to investigate the effect of COUP-TFII over- and under-expression on the ability of mesenchyme to induce epithelial differentiation in kidney capsule, and the growth rate of prostate tumors induced by epithelial cells in athymic nude mice. This study will provide us important insights for possible roles of COUP-TFII during the development of the prostate and the tumor progression of prostate cancer. It may raise the possibility that COUP-TFII and its regulated-signal molecules could be used as targets to establish novel therapeutic strategies for the improvement of diagnosis, prevention, prognosis and treatment of human prostate cancer and other forms of malignancies.

Specific Aims:

Aim 1. Effect of COUP-TF over- or under-expression on the ability of mesenchyme to induce epithelial differentiation in kidney capsule. We will construct adenoviral vectors capable of over-expressing or under-expressing COUP-TFII by expressing sense or antisense COUP-TFII messenger RNA. These adenovirus expression vectors will be used to express COUP-TFII in isolated primary mesenchymal cells from the urogenital sinus. The resulting cells will then be reconstituted with isolated prostate epithelial cells in the kidney capsule to study their effect on prostate epithelial differentiation.

Aim 2. Effect of over- and under-expression of COUP-TFII on the growth rate of prostate tumors induced by epithelial cells in athymic nude mice. We will develop vectors capable of over-expressing or under-expressing COUP-TFII by expressing sense, antisense, or dominant-negative COUP-TFII messenger RNA, and generate stable rUGM cell lines with these constructs. Once these cell lines are established, we will introduce them together with prostate epithelial cells into male athymic nude mice and assess the effect of COUP-TFII expression on the growth rate and differentiation of resulting tumors.

Key Research Accomplishments:

Construction of Adenoviral Vectors for Over- and Under-expressingof COUP-TFII mRNA

To achieve the goals of over- and under-expression of COUP-TFII in primary mesenchymal cells from the urogenital sinus, we choose to generate the high-capacity adenoviral vector (HC-Ad) that carries the mouse COUP-TFII sense and antisense cDNA under control of the cytomegalovirus (CMV) early promoter to deliver DNA to the majority of cells. This system was developed by Dr. Stefan Kochanek in Dr. Thomas Caskey's laboratory (5), and later modified by Dr. Frank L. Graham's laboratory (6).

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Since the vector contains no viral coding sequences, it possesses a very large insert capacity (up to 35 kilobases) and greatly reduces the cellular immune response triggered by the expression of viral proteins.

The polylinker between BglII and HindIII sites in viral plasmid (pAvCvSv) was replaced by either the sequence 5'-ATCTCTGCAGGCGGCCGCGATATCGGTACC-3' or 5'-ATCTCTGCAGGGTACCGATATCGCGGCCGCA-3'. Both inserted sequences contain NotI and KpnI sites, which were used for ligation of a fragment containing mouse COUP-TFII cDNA from pBluescript II KS+. The resultant vectors were named pAv-COUP-TFII-NK and pAv-COUP-TFII-KN, respectively (Fig.1), and then delivered into 293Cre4 cells to produce adenoviral particles.

Preparation of Adenovirus

Adenoviral constructs were transfected into 293Cre-4 cells. Subsequently, the cells were infected with LoxP helper virus AdLC8cluc (6). To increase the titer, vector lysates were passed through 293Cre4 cells several times. Freeze/thaw two times to lyse the cells and release the virus to get crude viral lysate (CVL). Cell debris was removed from CVL using ultra-clear Beckman #344060 ultra-centrifuge tubes (SW40 rotor, 10,000 rpm, 4^0C, 5 min). The remaining helper viruses were separated by double CsCl equilibrium density centrifugation. The detailed procedure for adenoviral rescue and virus characterization followed that described by Schiedner et al. (7). The concentration of the viral particles was ≈4-7 x 10^{11}/ml. The particle/infectious units' ratio was 20:1. The contamination of Lox-P helper virus in the virus preparation was ≈0.01-0.05%. In addition, the viral preparation did not contain any replication competent adenoviruses.

Test of the Expression Efficiencies of COUP-TFII Sense and Antisense Adenoviral Vectors

It has been shown that COUP-TFII is able to activate NGFI-A expression in both Hela and rat urogenital mesenchymal (rUGM) cells (8). To test whether the constructed adenoviral vectors are able to efficiently express sense and antisense COUP-TFII , NGFI-

A-luciferase construct (8) were used as reporter. After transfection, cells were infected with 10^7 to 10^9 particles/ml of virus for 1 hour. 48 hours later, the amount of luciferase activity was measured to determine the expression level of COUP-TFII. As shown in Fig 2, more than 10-fold induction of promoter activity was observed when 10^8 to 10^9 of sense virus/ml were infected. When similar amount of antisense virus were infected, 90% of COUP-TFII-induecd promoter activity was abolished. These data strongly suggested that adenovirus indeed efficiently express both sense and antisense COUP-TFII mRNA. These virus are being used to analyze the effect of COUP-TFII over- or under-expression on the ability of mesenchyme to induce epithelial differentiation in reconstitution system of kidney capsule.

Establishment of Rat Urogenital Mesenchymal Cell Lines Which Over- and Under-Express COUP-TFII mRNA

The rUGM cell lines (9) were grown in T medium (2) supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin. Ten-centimeter dishes of rUGM cells were transfected by lipofectin with plasmids pCNX containing the neomycin resistance gene and the COUP-TFII open reading frame in right or reverse orientation. Cells were then cultured in the medium containing 300 μg of Geneticin/ml for 2 weeks, and colonies were picked and expanded. The expression level of COUP-TFII were examined by Western and Northern blotting.

Ongoing Experiments

Since we have obtained adenovirus overexpressing sense and antisense COUP-TFII gene, we are testing their abilities to infect mesenchymal cells. After we ascertain that majority of cells express sense or antisense COUP-TFII, we will use these infected cells for reconstitution with isolated epithelial cells in a medium containing collagen and later grafted under the renal capsule of isogenic male adult hosts (3). Three to five weeks after grafting into the kidney capsule, the graft will be removed and analyzed histologically and immunologically. The questions that we want to address are: 1) whether over-

expression of COUP-TFII changes the pattern and timing of prostate differentiation; 2) whether the reconstituted prostate differentiate in the absence or depletion of COUP-TFII; 3) whether PSA, cytokeratin, vimentin and desmin expression are induced in this reconstituted prostate; and 4) whether differentiation of the reconstituted prostate is androgen-dependent. Besides, We also infected prostate cancer cell line DU145 with the adenovirus expressing sense and antisense COUP-TFII mRNA, and injected them into nude mice. The growth rate, tumor volume, histological and immunological properties of the tumors formed are being analyzed. Finally, we are planning to mix the established rUGM cell lines which over-express COUP-TFII (sense) or under-express COUP-TFII (antisense) with prostate epithelial cell lines that are either androgen positive (NbE-1 and LNCaP) (2, 10, 11) or androgen negative (PC-3) or the control bladder epithelial cell line (WH) (11) and injected s.c.into male athymic nude mice. Their effect on the tumorigenesis will be evaluated by studying tumor growth rate, androgen- dependency, and histological and immunological properties of the resulting tumors.

Figure Legend

FIG. 1: Structure of pAV-COUP-TFII sense and antisense constructs. The constructs contain the left terminus of adenovirus type 5, a fragment of the human hypoxanthine-guanine phophoribosyltransferase gene, CMV promoter, SV40 poly(A), and COUP-TFII cDNA in a right or reverse orientation.

FIG. 2: Adenoviruses efficiently express both sense and antisense COUP-TFII mRNA. A luciferase reporter plasmid (250 ng) containing 1.4 kb of the rat *NGFI*-A promoter region was transfected along with COUP-TFII expression vector (0.25 μg), 10^8 of sense and/or antisense virus particles. Cells were lysed in 200μl of lysis buffer, and 20-μl volumes of the extracts were assayed for luciferase activity

pAV-COUP-TFII Sense and Antisense Vector

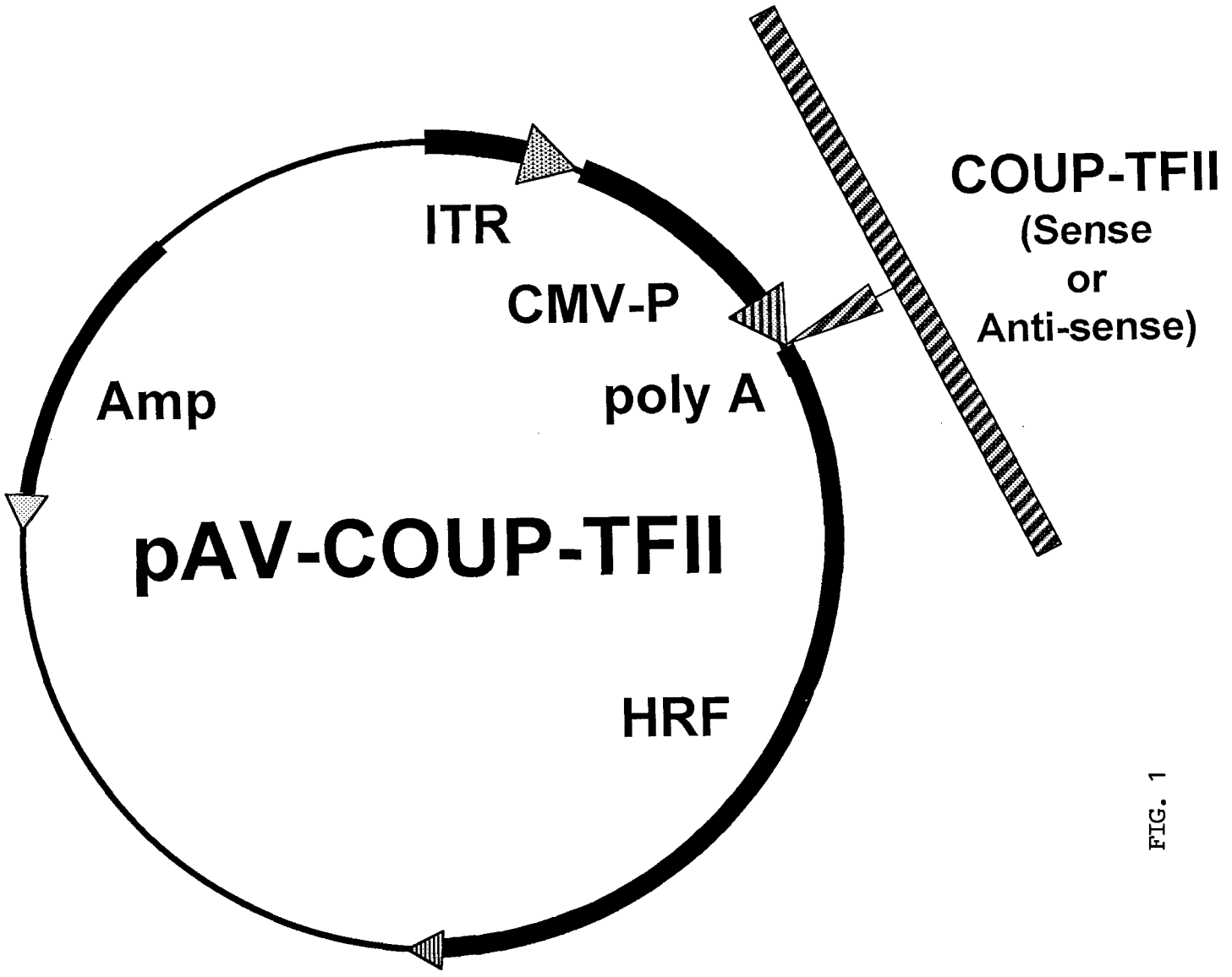

FIG. 1

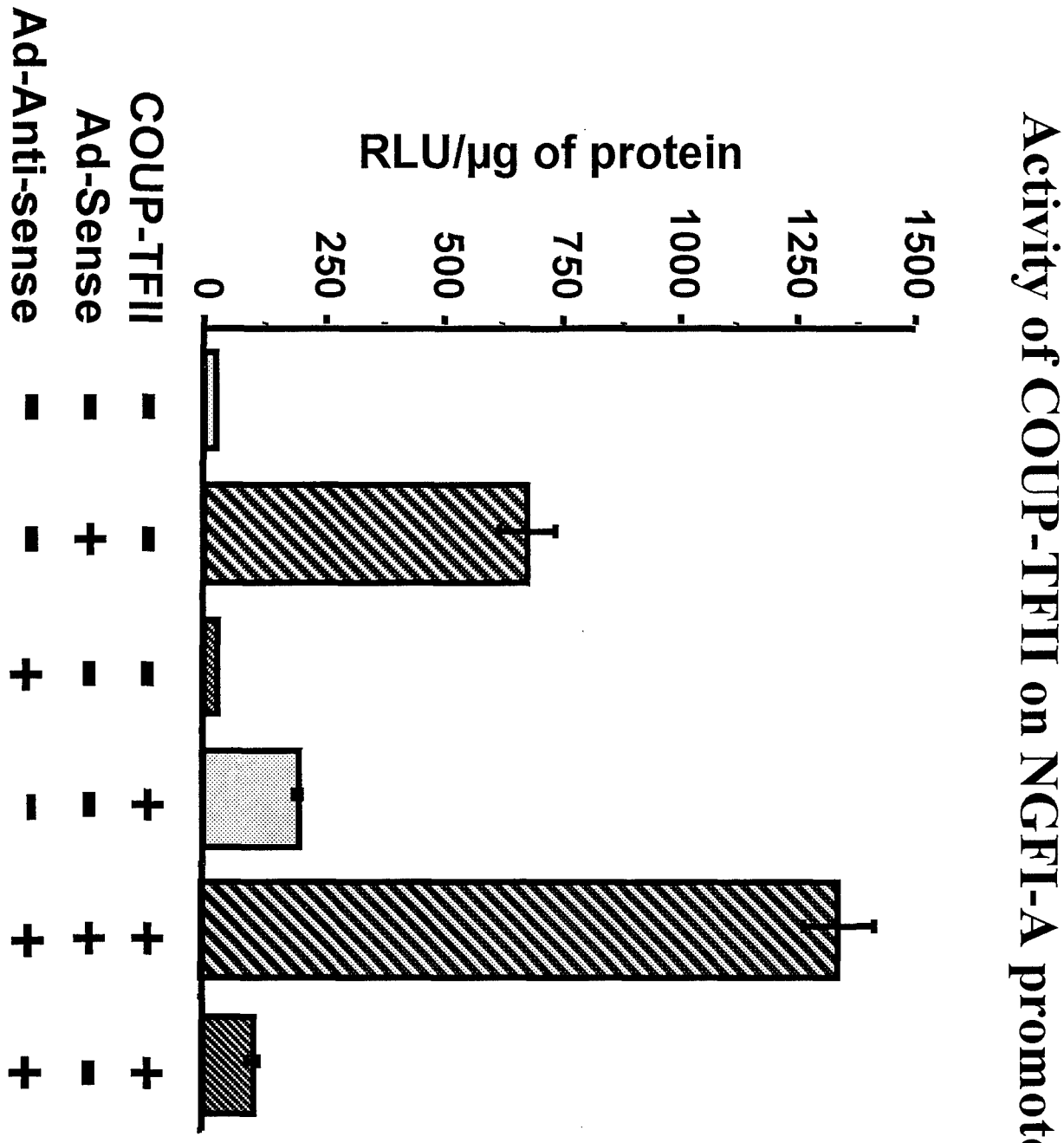
Activity of COUP-TFII on NGFI-A promoter
RLU/µg of protein
0
250
500
750
1000
1250
1500
COUP-TFII
Ad-Sense
Ad-Anti-sense
- - -
- + -
- - +
+ - +
+ + +
+ - +

FIG. 2

Reportable Outcomes

None

Conclusions

As summarized above, we have made major progress toward our goals. We have successfully generated adenoviral vectors that effectively express sense and antisense COUP-TFII. More effort will be devoted to analyze the effect of COUP-TFII over- or under-express on the ability of mesenchyme to induce epithelial differentiation in reconstitution system of kidney capsule, as well as on the ability of tumorigenesis of prostate cancer cell lines in nude mice. In addition, we established rUGM cell lines which over- and under-express COUP-TFII. The effect of changing the level of COUP-TFII expression in rUGM cells on the modulation of tumor growth induced by epithelial cells in asthymic nude mice is being studied.

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Appendices

Key research accomplishment:

1. Construction of Adenoviral Vectors for Over- and Under-expressingof COUP-TFII mRNA;
2. Preparation of Adenovirus;
3. Test of the Expression Efficiencies of COUP-TFII Sense and Antisense Adenoviral Vectors;
4. Establishment of Rat Urogenital Mesenchymal Cell Lines Which Over- and Under-Express COUP-TFII mRNA.

Reportable Outcomes

None